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Identification of the skin virome in a young boy with widespread HPV2-positive warts that completely regressed after administration of tetravalent human papillomavirus vaccine

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DEAR EDITOR, Human papillomavirus (HPV) infections are normally controlled by an intact cell-mediated and humoral immune system. Patients with cell-mediated immunodeficiencies, whether primary, secondary or iatrogenic are all, therefore, at increased risk of developing extensive, persistent and recurrent warts.¹ The sequestration of HPV in epithelial cells can however provide protection for the virus, resulting in inefficient activation of innate immunity, poor priming of the adaptive response and permits infections to persist, even in immunocompetent individuals.² In addition, genome variations, especially in the LCR and E2 ORF, which enhance viral promoter activities, have been reported and associated with enhanced viral replication and unusual clinical manifestations.³⁻⁵

A 16-year-old boy presented in October 2012 with a 4-year history of recalcitrant multiple large, hyperkeratotic, fissured cauliflower-like plantar warts on the right foot. Some small warts were also developing on his hands (Fig. 1, upper row). He had no relevant personal or family history of other illnesses or dermatologic conditions and was HIV negative. He had previously been treated with numerous therapies, including keratolytic, diathermic, cryotherapeutic, photodynamic, and topical imiquimod without any improvement. Since the warts kept enlarging and spreading, affecting the patient's quality life, we decided to treat the patient with intravenous cidofovir (5 mg/kg for 5 cycles with one week intervals). The treatment was well tolerated, but with no sign of wart regression 3 months post-treatment. Immunophenotype analysis of the patient's peripheral blood mononuclear cells failed to reveal any abnormalities and immunoglobulin levels were also shown to lie within the normal range.

The DNA extracted from swabs obtained from the skin overlying the plantar warts, from unaffected sites (pooled from different sites and from the forehead separately), and from plucked eyebrows was analyzed by PCR for α - and β -HPV genotypes.⁶ Positivity for α -types was found for the pooled unaffected skin swabs and for the plantar warts.

Biopsies from the plantar warts were available as formalin-fixed paraffin-embedded (FFPE) blocks. Since detection of the anti-E4 protein provides a well-established biomarker of productive

HPV infection, tissue sections from these blocks were stained by immunofluorescence (IF) using a panel of in house anti-E4 antibodies against the cutaneous genotypes $\alpha 2$, $\mu 1$ and $\gamma 4$.⁷ As shown in the lower panels of Fig. 1, only anti-E4-HPV2 gave a cytoplasmic staining in the more superficial layers. HPV2 infection, whose reported prevalence in multiple common warts ranges between 28 to 34% in the European population,⁸ was confirmed by fluorescence *in situ* hybridization (FISH), using the HPV2 genome as a probe, with many cells staining positive for both nuclear HPV2 and cytoplasmic E4 (Fig. 1). Consistent with these findings, HPV2 specific real time qPCR (see Supplementary Material and Methods) revealed very high viral loads (up to 417 copies/cell) in skin swabs from the plantar warts as well as in the unaffected skin sites (180 copies/cell). According to the clinical presentation, the histology of the warts showed prominent papillomatosis, acanthosis, hypergranulosis, and hyperkeratosis of the horny layer. A marked clearing of the cytoplasm in granular cells ('koilocytosis') and numerous keratohyalin granules were also present (Fig. 1, HE).

On the basis of: i) sporadic literature reports on the cross-protective potential of the quadrivalent HPV vaccine (types 6, 11, 16, and 18);⁹⁻¹² ii) 65% identity between the L1 protein from HPV2 and the vaccine types, which supports a possible low-level cross-protection;¹³ and iii) the failure of conventional therapies, the decision was taken to vaccinate the patient. Dosage was administered in the patient's arm in 3 shots (the first in August 2013, the second in October, and the third in February 2014). Improvement was noticed as early as 6 weeks after receiving the first injection. When the patient returned for a follow-up assessment in November 2013, the warts had significantly decreased in size and thickness, and by March 2014 they had completely regressed. Accordingly, the viral load values of skin swabs from the plantar region had also dropped to very low levels (2 copies/cell).

To gain more insight into the patient's skin viral microbiota,¹⁴ DNA extracted from skin swabs from the affected foot was rolling-circle-amplified and deep sequenced (see Supplementary Material and Methods). Sequencing reads were cleaned to remove any human sequences and compared to genomes available in the PapillomaVirus Episteme (PaVE) database

(<http://pave.niaid.nih.gov/>). Those that could not be assigned to HPV genomes were further compared to whole viral and bacterial genome datasets available in the NCBI databases. Thirty percent of all reads (45,343) could be assigned to HPV genomes. A very small fraction (156 reads) displayed matches with nonHPV viral genomes - although it was not possible to make any taxonomic assignment as the similarity and coverage did not satisfy the chosen thresholds. A significant fraction (30,773 sequences, 20%) matched bacterial sequences. All sequences matching HPV genomes displayed best alignment with HPV2 genomes. Interestingly, more than 95% of the HPV2-related reads showed the presence of 7 nucleotide substitutions (when compared with the reference sequence; Table 1); including one point mutation in the LCR region (nt7720) and a novel amino acid exchange (V:A) in the E2 protein.

Resolution of skin warts by the HPV vaccine has previously been reported in a small number of case studies,⁹⁻¹² but this study is the first to perform a comprehensive skin virome analysis of the patient. The abundance of HPV2 reads, together with the lack of viral DNA of other common constituents of the human skin microbiome (including β -HPV and skin tropic HPyV), indicates that the viral skin flora can be significantly affected by the presence of widespread productive skin lesions.¹⁴ The observed genomic variations in the LCR and E2 regions of the HPV2 isolate, including a novel amino acid exchange in the E2 ORF (aa335), may contribute to the unusual clinical manifestation of the infection. The mutation found in the LCR region has previously been reported in patients with multiple warts and extensive cutaneous horns, and has been associated with a stronger promoter activity.³⁻⁵

Finally, although we cannot exclude the possibility that the resolution of the patient's warts following vaccine administration was coincidental, the observations from this case report warrant further studies to support the therapeutic use of the tetravalent vaccine in HPV2-induced recalcitrant warts in immunocompetent individuals.

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FIGURE LEGEND

Fig. 1. Photographs of the affected skin areas and distribution of the viral E4, L1 protein and HPV DNA in biopsies from plantar warts of the right foot from the study patient. The photographs in the upper row show some small warts on the patient's left hand and the hyperkeratotic, fissured cauliflower-like plantar warts on the patient's right foot. The photographs presented in the second row show the same affected areas after having received the HPV vaccine: the two images on the left hand side were taken 1 month after the second administration, while the two images on the right hand side were taken three weeks after the third administration. The left hand picture in the third row shows the HE staining pattern of a biopsy section of one of the warts shown above in the upper rows. In the central image, the same section was stained for HPV2 DNA using FISH (red) to visualize the cells in which viral genome amplification was occurring. The right hand image shows a serial section co-stained with antibodies for the late capsid protein L1 (red) and the early viral protein E4 (green). The white dotted line indicates the position of the basal layer. All sections were counterstained with DAPI (blue) to visualize cell nuclei. Scale bar = 200 μ m.

Table I. Nucleotide mutations and their location in the HPV2 genome isolated from the study patient

Nucleotide		Amino Acid		ORF	References
Position	Substitution	Position	Substitution		
1936	A:G	375	conserved, Q	E1	Wang et al., J Clin Virol 2007 Lei et al., Intervirology 2007
2167	T:C	452	conserved, A	E1	this work
3688*	T:C	335	V → A	E2	this work
4156	G:T	-	-	-	Wang et al., J Clin Virol 2007 Lei et al., Intervirology 2007
4471	A:G	79	conserved, A	L2	this work
5626	C:T	464	conserved, T	L2	Wang et al., J Clin Virol 2007 Lei et al., Intervirology 2007
6565	T:G	269	L → R	L1	Wang et al., J Clin Virol 2007 Lei et al., Intervirology 2007
7720	G:C	-	-	LCR	Wang et al., J Clin Virol 2007 Lei et al., Intervirology 2007 Chan et al., Virology 1997

* this mutation was observed in ~30% of the reads, while all other mutations were present in at least 97%



Landini et al., Figure 1